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Published to advance the Science of cold-blooded vertebrates

NOTES ON THE PRESERVATION OF THE COLORS OF FISHES.

Probably every collector of brightly colored fishes has wondered if there was some way by which the natural colors could be preserved. It must be admitted at the beginning that we have not found a universal preservative for the colors of fishes, but the data presented herewith may be an inspiration to further investigation along these lines. The true test of permanency is, of course, indeterminate, since the specimen must retain its colors indefinitely. However, the results so far obtained will be of value to collectors who desire to make small collections of fishes with the intention of making colored drawings within a period of several weeks afterward. The advantages of thus being able to inhibit the fading of the colors for even a short time seem obvious, especially on long expeditions where it is not always feasible to take color notes from living specimens in the field.

Before setting forth the methods whereby the colors of fish may be preserved, it is necessary to understand the mechanism of the coloration of fishes. There is a great deal of literature on the subject, chiefly by European workers. The most notable works by American authors are those of Mast

(1914)¹ and Kuntz (1916)². The former work deals with the changes in shade, color and pattern of fishes, while the latter furnishes a histological basis for these changes.

The colors of fishes seem to be of two kinds: (1), those which are due to pigment in the chromatophores in the skin, and (2), interference colors which are due to combinations of the optical effects caused by the pigment in relation to other special cells known as guanophores. The presence or absence of large numbers of guanophores determine whether the shade is dark or light. To the first group belong such colors as black, brown, red, orange, yellow, green and blue with intermediate colors and shades. The second group includes the iridescent effects and other colors not due directly to the pigment in the chromatophores.

It has been clearly demonstrated that the chromatophores are under the control of the sympathetic nervous system. Kuntz (1916) has shown that there is no change in the shape of chromatophores in adult fishes, although there are well-known amoeboid movements of the chromatophores in young fishes. The pigment is in granules which stream from or toward the center of the cell as the adaptation is to dark or light shades. The various pigments are known as melanin which varies in color from black to brown; xanthin which varies from yellow to orange or red; and in some fishes, cyanin, varying from green to blue. The chromatophores are named by the pigment which they contain, viz., melanophores, xanthophores and cyanophores. The actual coloring matter is a fatty substance or lipochrome. Some of

¹ Mast, S. O. Changes in shade, color and pattern in fishes, and their bearing on the problems of adaptation and behavior, with special reference to the flounders, *Paralichthys* and *Ancylopussetta*. Bull. U. S. Bureau of Fisheries, 1914 (1916). Vol. XXXIV, p. 173-238. Doc. 821, issued May 10, 1916.

² Kuntz, Albert. The Histological basis of adaptive shades and colors in the flounder, *Paralichthys albiguttus*. Bull. U. S. Bureau of Fisheries, 1915-16 (1918). Vol. XXXV. 29 pages. Doc. 842, issued May 28, 1917.

these lipochromes may be converted from green to red by the application of heat, or in the presence of alcohol.

The exact chemical composition of these pigments is not known, but they are supposed to be related to excretory products. Xanthine is a white crystalline compound ($C_5H_4N_4O_2$) which is contained in the blood, urine and other animal secretions. The yellow color of fishes does not seem to be xanthic oxide, the yellow coloring matter in flowers, since this compound is known to be insoluble, while it is known that the yellow color in fishes quickly fades in alcohol resulting in a colored solution. In all of the various solutions with which experiments have been made it is apparent that blues and greens persist for a much longer time than the other pigments, except possibly black.

Although little seems to be known about the chemical composition of the pigments, it nevertheless is possible to find by experiment some solution in which they are only slightly soluble. The problem is thus to find a solution in which the pigments will not dissolve and which at the same time will prohibit bacterial or other decomposition. The well-known effects of alcohol and formaldehyde on the colors of fishes at once precludes their use in unmodified solutions.

The colors may be preserved fairly well in solutions of various salts. Among these may be mentioned ammonium nitrate, chloride, and acetate; sodium nitrate, chloride, acetate and benzoate; and the corresponding salts of potassium. The specimens are dry salted and allowed to become quite hard. The use of sodium benzoate in small quantities is sufficient to prohibit bacterial action and the colors remain fairly well preserved.

The following formula published a number of years ago in the Scientific American under the heading, "Preserving bodies in their natural form and

color," as employed by G. E. Weise, has been experimented with.

600 g. of sodium hyposulphite dissolved in 5,000 g. water.

75 g. of ammonium chloride dissolved in 250 g. water.

These two solutions are mixed and 4 to 6 litres of grain alcohol are added. Specimens of the European pearl roach, *Scardinius erythrophthalmus* (Linn.) and the common gold fish, *Carassius auratus* (Linn.) were placed in this solution alive and after two weeks it was noticed that the solution had become slightly colored, although there was no apparent change in the colors of the specimens. Little or no shrinkage was observed and the fin membranes remained transparent. This solution can be recommended for a short time, or for short field trips.

Various modifications of this solution were tried, such as adding 950 cc. water and 50 cc. of 40% formaldehyde instead of the alcohol, and then adding various salts. None of these solutions seem to be superior to the first one given, although additions of sodium nitrate seemed to inhibit fading somewhat more than was the case with other salts. These solutions all threw down a precipitate of sulphur which, being inert, was detrimental in no way. Specimens of pearl roach treated in the solution modified by formalin and sodium nitrate retained their colors almost unimpaired for ten days after which it was noticed that the red color in the fins slowly faded, but no color was imparted to the solution. After 39 days the red had faded to orange and yellow while specimens in other solutions had faded much more rapidly and by this time showed only a faint indication of the former color. It was noticed that the more formaldehyde present in the solution the quicker the colors faded.

Common salt (NaCl) is an efficient color preservative when the specimens are dry-salted with it, but

the disadvantages are that salt is heavy and bulky and does not preserve the specimens indefinitely, although if they are properly cared for salted specimens may last several months. If they are then soaked in fresh water until the salt is removed and then placed in formal alcohol, the colors may be retained for several weeks, the reds and yellows fading first.

Sodium nitrate (NaNO_3) is better than sodium chloride, but its use is accompanied by similar disadvantages. Potassium nitrate is more expensive and offers no additional compensations.

Ammonium acetate was recommended by Dr. Theodore Gill many years ago. The specimens become hard and shrunken, but when they are soaked in fresh water the tissues assume about their original bulk and the colors are remarkably preserved.

Our experiments have been impeded by lack of material. How much variation, if any, exists between different species of fishes still remains to be determined. It is not known at this time whether the red or green colors in one fish are identical to the same colors in other fishes in their reactions to our solutions. The indications are that the pigments are soluble to some extent in a great variety of solutions either at once, or if not, are rendered soluble by enzymic decomposition which is inherent in the fish. It would, therefore, seem that no solution will preserve all of the colors indefinitely, although the time it takes them to fade much may be greatly prolonged.

The requirements which must be met by any permanent preservative are:—(1). A solution which will not dissolve the pigment from the chromatophores or cause it to change color. (2). Something that will not disturb the relationship of the chromatophores and guanophores at the moment of the death of the fish. (3). Preservation of the tissues and viscera of the fish. All of these things depend upon a more intimate knowledge of the chemistry of the col-

ors of fishes and the attempt to solve these problems may open up a very interesting field of study.

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A FURTHER NOTE ON SNAKES SWALLOWING THEIR YOUNG

My remarks on the problem of snakes swallowing their young for protection (1921, *Copeia*, No. 98, p. 54), have brought forth further discussion. The several interesting questions raised by Pope (1922, *Copeia*, No. 102, p. 6), have been considered by previous writers and have been answered by them in detail. References to these accounts appear in my earlier note. * Recently a further circumstantial account has been brought to my attention by Mr. C. L. Camp. This is a detailed statement of observations on two specimens of *Crotalus atrox* which were observed to swallow their young. Although the observations were made by Mitchell, a herpetologist of note, they were made years before the account was published (Mitchell, 1903, Trans. Texas Acad. Sci. (for 1902), Vol. V, part 1, pp. 36-37) and "undoubted proof" may be said to be lacking. The Editors of *Copeia* still look forward to the receipt of such proof as specimens preserved with the young *in situ*.

G. K. NOBLE,
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* Unfortunately, this number of *Copeia* was not edited with as much care as usual and many typographical errors appear. These are too numerous to correct here.